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ON THE PROTOZOA PARASITIC IN FROGS*

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Probably no other animals have been for many years more favorite objects of studies by zoologists than the frogs. The amphibians have been examined by several Protozoologists and we know at present a considerable number of Protozoa of a great variety parasitic in frogs from various parts of the world.

Numerous publications dealing with the protozoan parasites of frogs have been issued by authors of several nationalities. Aside from the papers by North American workers such as Ohlmacher (1893), Whinery (1893), Gurley (1894), Stebbins (1904, 1905), Lewis and Williams (1905), Metcalf (1909) and Swezy (1915, 1915a), a large majority are widely scattered in various periodicals, and are not always easily referred to. Undoubtedly this hardship concerning literature prevented the students in Zoology from taking advantage of the material. If one possesses therefore brief accounts of the Protozoa commonly found in frogs, hundreds of which are sacrificed yearly by students in Zoology and by special investigators, one can utilize both material and time in carrying out observations upon these interesting Protozoa.

The present paper is an attempt to meet this need. It deals with my observations on the Protozoa parasitic in North American frogs which I have examined during the last two years, together with the description of methods of observation, and with brief review of and reference to the works of the previous investigators on the subject.

The following six species are described in order:

1. *Entamoeba ranarum* from the intestine
2. *Leptotheca ohlmacheri* from the kidney
3. *Haemogregarina* sp. from the blood

*Contributions from the Zoological Laboratory of University of Illinois. No. 199.

4. *Trypanosoma rotatorium* from the blood
5. *Trypanosoma parvum* nov. spec. from the blood
6. *Opalina* sp. from the intestine

I *Entamoeba ranarum* (Grassi) Dobell 1908

Habitat.—In the large intestine of *Rana temporaria*, *R. esculenta*, *R. clamitans* and *Bufo vulgaris*. Dobell (1909) saw that about 23% of *Rana temporaria* in Cambridge and Munich, were infected. I have seen a number of amoebae whose characters agree on the whole with those described by Dobell for *Entamoeba ranarum* in one out of 14 individuals of *Rana clamitans* from New York in August of 1920. In *Rana pipiens* which I have studied in 1920 and 1921 at Urbana, Illinois, I did not observe any host individual that harbored the organism. This of course does not mean its absence in a frog of this species, since I have not examined them as thoroughly as I did in the case of *Rana clamitans*.

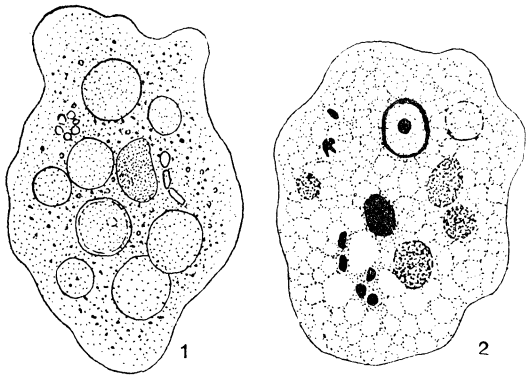
Historical.—Lieberkühn (1854) probably noticed the Amoeba in the intestine of the frog which he studied. Grassi (1879) examined and named it *Amoeba ranarum*. Dobell (1909) found an Amoeba in the frogs of England and Germany, and studied them in detail. Quite recently, the same author (Dobell, 1918) states that although the Amoeba resembles closely morphologically to *Entamoeba histolytica* of human intestine, they are distinct species. I have met with apparently the same Protozoon but once, and could not carry observation concerning its development.

Distribution.—Europe and North America.

Methods of observation.—A portion of the large intestine of a frog is cut into small pieces in physiological salt solution on a cover-glass, made an ordinary fresh preparation and observed. The organism may live for several hours. The general appearance, changes in form of the body through the formation of pseudopodia and the structure of the protoplasm can be studied. To make permanent preparations, make smears on slides or cover-glasses and fix them with hot sublimate-alcohol-acetic mixture (2 parts of saturated aqueous solution of corrosive sublimate, 1 part of absolute alcohol and 5% of glacial acetic acid) for about 20 minutes. The smears are then immersed for about 15 minutes in a weak iodine alcohol (50%) and then transferred into a plain alcohol to remove the iodine. Staining with Delafield's haematoxylin, Heidenhain's iron haematoxylin or Dobell's alcoholic haematein, brings out satisfactory results.

Morphology.—Amoeba of moderate size. When alive, the cytoplasm is poorly differentiated into ectoplasm and endoplasm. Lobose pseudopodia are actively formed at one time from any part of the body. The peripheral portion of the cytoplasm is somewhat hyaline, while the main part of the body is granulated and contains bacteria, yeasts and other particles

present in the host intestine. The nucleus is spherical and faintly visible in living condition with an oil immersion objective. No contractile vacuole is present. Dimensions vary from 15 to 40μ in the largest diameter. When stained, the cytoplasm becomes highly vacuolated or reticulated. The nucleus is spherical and usually contains a distinct karyosome.



Figs. 1 and 2. *Entamoeba ranarum*. Fig. 1, a living individual. Fig. 2, an individual stained with Delafield. x 1500.

Development.—According to Dobell, the cysts are found in the host intestine in winter months. They are spherical, and measure 10 to 16μ in diameter with a large nucleus. The nucleus divides twice producing four daughter nuclei. Further changes are not known. Dobell suggests that the cysts serve for the dissemination of the organism. The same author (Dobell, 1918) recently found that although *Entamoeba ranarum* and *E. histolytica* can hardly be distinguished morphologically from each other, the cysts of the latter species when introduced into the intestine of tadpoles did not undergo changes which take place in their proper habitat, and concluded that these two forms should be held as different species.

II *Leptotheca ohlmacheri* (Gurley) Labbé 1899

Synonyms.—*Chloromyxum* (*Sphaerospora*) *ohlmacheri* Gurley 1893, *Leptotheca ranae* Thélohan 1895 and *Wardia ohlmacheri* Kudo 1920.

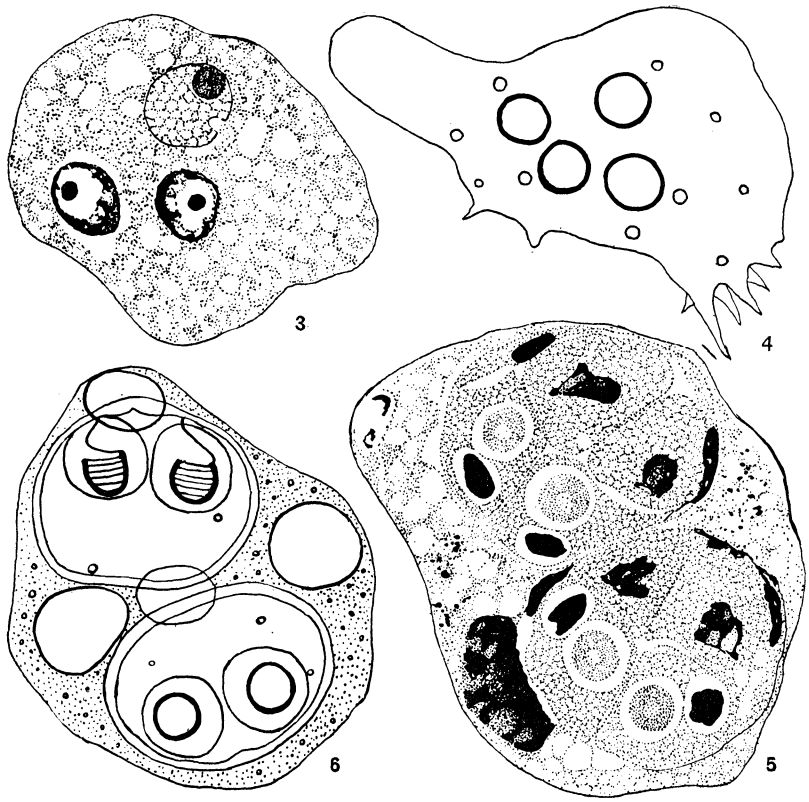
Habitat.—In the kidney of *Rana clamitans*, *R. pipiens* and *Bufo lentiginosus*. Out of 14 individuals of *Rana clamitans* examined in New York from July to September, 1920, six were infected by the parasite. Out of 24 *Rana pipiens* bought from a Chicago biological supply store and examined between November and December, 1920, ten were found to be infected by the Myxosporidian. Thélohan (1895) named a Myxosporidian which he saw in the kidneys of *Rana esculenta* and *R. temporaria*, *Leptotheca ranae*. He has not given description nor figure for it, but I am inclined to think this is probably identical with the American species.

Historical.—The spores of the Myxosporidian were first found by Ohlmacher (1893). Whinery (1893) also worked on them. Gurley (1894) summarized the observations of the two authors. Thélohan (1895) found *Leptotheca ranae* in France (?). No body seemed to have worked on the organism until 1920 when I found the vegetative stages and spores of a species what appeared to be identical with the present form. I have studied its morphology and development, the result of which will be stated elsewhere (Kudo, 1922).

Distribution.—North America and Europe (?).

Methods of observation.—When the infection is heavy, isolated spores may be found in the cloaca of the host, but the kidney must be examined for both spores and trophozoites. A small part of the kidney is cut into small pieces on a slide in a drop of physiological salt solution and made fresh preparation. In order to remove the fat globules that are usually present in smears of kidneys, one drop of weak potassium hydrate solution may be added to it. If any spores are present, they will be easily recognized under a low power due to their peculiar appearance. If the infection of the kidney is detected, hanging drop preparations or fresh preparations with physiological solution should be made immediately and sealed with melted paraffin. By using oil immersion objective, one can distinctly see the detailed structures of the spores and trophozoites of various developmental phases. To make permanent preparations, smears of variable thickness should be made. In thinly made portion, one can see the number and structure of the nuclei in well stretched trophozoites, while in thickly made part, the shape, general appearance and arrangement of nuclei and cytoplasm around them may be studied. Smears are well fixed with sublimate-alcohol-acetic mixture. For staining, besides the three methods stated for *Entamoeba ranarum*, Giemsa's method brings out beautiful results. Section preparations should also be made in order to observe the location of various developmental stages of parasites in the host organ and the relation between the parasite and host body.

Morphology.—Fully grown trophozoites are usually rounded or oval in form. Long conical pseudopodia are actively formed. Frequently the trophozoites are completely rounded without any pseudopodia. The body is colorless, granulated and extremely hyaline. The cytoplasm is indistinctly differentiated into endoplasm and ectoplasm. The endoplasm is finely granualted and contains a vegetative nucleus, two developing spores and fat globules of variable size and number. The ectoplasm is only distinctly visible where the pseudopodia are formed, the latter being usually entirely composed of ectoplasm. Before starting spore formation the trophozoites multiply by active gemmation. In almost all cases, disporous, rarely monosporous and more rarely trisporous.



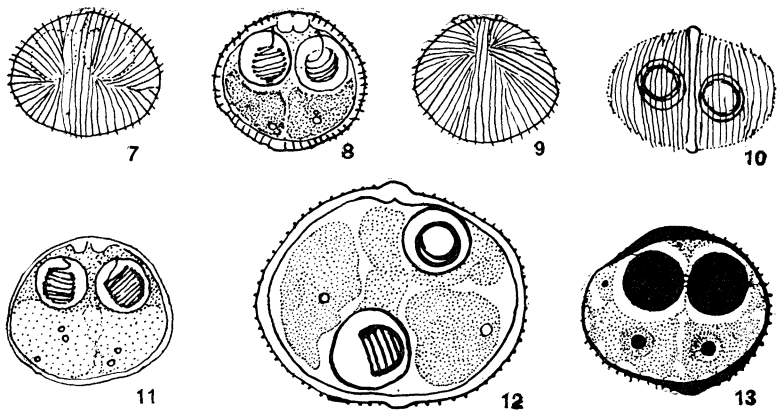
Figs. 3 to 6. Trophozoites of *Leptotheca ohlmacheri*. Fig. 3, a trinucleate trophozoite with a vegetative nucleus and two generative nuclei. A thin smear stained with Delafield. Fig. 4, a young trophozoite in which four polar capsules are being formed: fresh preparation. Fig. 5, a thinly spread trophozoite with a vegetative nucleus and two developing spores. Giemsa. Fig. 6, a rounded trophozoite with two mature spores: fresh preparation. All x 2350.

Dimensions of fully grown disporous trophozoites are 30 by 20μ, 38 by 25μ, 40 by 20μ, etc. Each spore develops independently.

Development.—With regard to the interesting development of the Myxosporidian the reader is referred to one of my papers (Kudo, 1922).

Morphology of the spore.—Oblong with its largest diameter standing at right angles to the sutural plane. Anterior end is conspicuously attenuated due to the thickening of the spore membrance at this point while the posterior end is rounded. In lateral view, it is nearly circular with a pointed anterior end. In an anterior end view, it is regularly oblong. The spore membrane is moderately thick. Sutural ridge is well marked, especially at the anterior end. The membrane is somewhat irregularly striated. Three to seven fine striae run parallel to the sutural

line on each valve and the remaining striae make somewhat similar angles with the sutural line. The striae in lateral view run parallel to one another except those on the posterior margin where a few make angles with the former. The striae on each valve vary from 25 to 35 in number.



Figs. 7 to 13. Spores of *Leptotheca ohlmacheri*. Figs. 7, 8 and 9, the upper surface, optical section and lower surface views of a normal fresh spore. Fig. 10, anterior end view of a fresh spore. Fig. 11, an optical section of a fresh spore with two large sporoplasms. Fig. 12, a fresh abnormal spore showing two sporoplasms and two capsulogenous cells, each containing a polar capsule. Fig. 13, a section through a spore showing the deeply stained polar capsules and two uninucleate sporoplasms. Section: Delafield. Figs. 7 to 11, x 1500; Figs. 12 and 13, x 2350.

Two polar capsules usually equal in size, occupy the anterior portion of the spore. The polar filament is coiled four to six times and is distinctly visible in fresh condition. It can be extruded under the action of potassium hydrate or mechanical pressure as I stated elsewhere (Kudo, 1918, 1921). Without staining the filament can be seen under a low magnification. Two independent sporoplasms occupy the extracapsular cavity of the spore, which condition is very rarely seen in Myxosporidia. They appear homogeneous in fresh state. Staining reveals that each sporoplasm contains a single nucleus. Dimensions of fresh spores: sutural diameter and thickness, 9.5 to 12 μ, breadth, 13 to 14.5 μ, diameter of polar capsules 3.5 to 4.5 μ, length of extruded polar filament, 42 to 62 μ. Those of stained spores: sutural diameter and thickness, 8.5 to 10 μ, breadth, 9 to 12 μ, diameter of polar capsules 3 to 4 μ.

III *Haemogregarina* sp.

Our knowledge of haemogregarines is still in great confusion because their development has not been studied except species occurring in reptiles. The haemogregarine described here seems to agree with the following

species: *Drepanidium magnum* Grassi et Feletti 1891, *Drepanidium krusei* Labbé 1892 and *Karyolysus clamatae* Stebbins 1905.

Habitat.—In the blood cell and plasma of *Rana clamitans* and *Rana pipiens*. I have observed it quite frequently in the last named host species. Quite frequently the frogs were found to harbor trypanosomes at the same time.

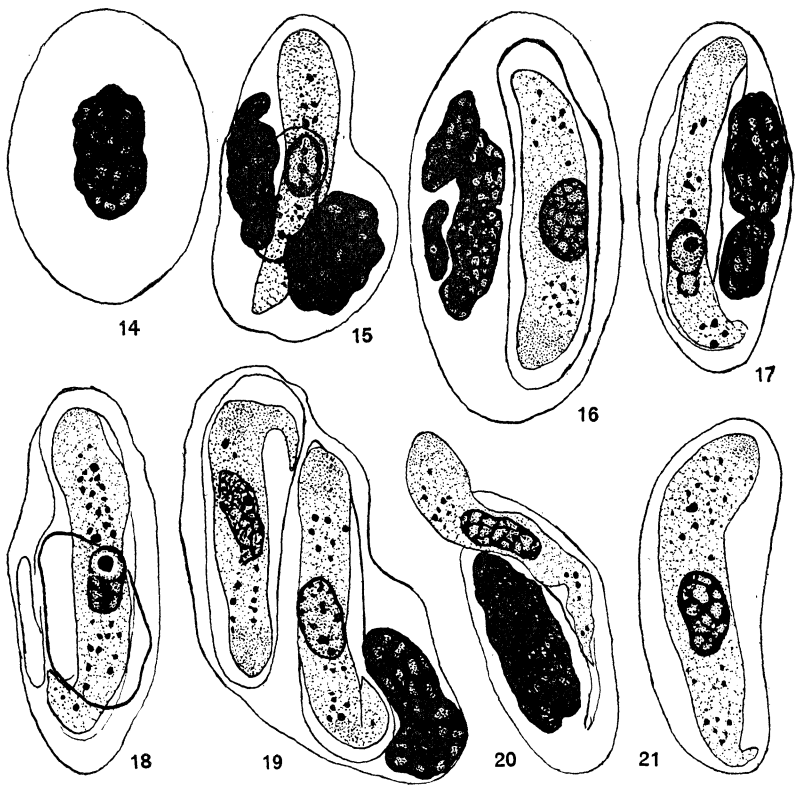
Historical.—While *Lankesterella minima* seems to have repeatedly been studied by European authors (see for instance, Hintze, 1902), the present form has been seen rarely. It seems to be this form that attracted the attention of some North American investigators such as Langmann (1898-1899) and Stebbins (1905). I have seen quite frequently the haemogregarine in frogs of New York and Illinois, but so far have not seen *Lankesterella minima*, the common European form.

Methods of observation.—Same as those stated for trypanosomes.

Morphology.—The haemogregarines found in the blood of frogs may be spoken of under two types: intracorpuscular and extracellular. The intracellular stage is cylindrical in shape, usually lying on one side of the erythrocyte and displacing the nucleus to the other side. The posterior end is usually folded up. In fresh preparation, the oblong nucleus with a distinct membrane and usually a single karyosome is seen to occupy the central portion of the body. The cytoplasm is homogeneous and contains refractive granules of variable number scattered both in the anterior and posterior regions of the body. Ordinarily there is no recognizable movements of the body except at the time prior to the emergence from the host cell in which the parasite is lodged. Around the body there is a thin but distinct membrane. When stained, the oblong nucleus assumes two kinds in appearance: one with eccentrically located karyosome and the other with chromatin granules scattered evenly on the linin network. The cytoplasm is highly reticulated and is always denser at the rounded end than at the other end. The nucleus of the host cell seems to degenerate by breaking down into a number of smaller irregular masses, and becomes faintly stained, which condition indicates that the infection probably causes some changes in the chemical nature of the nucleus of the host cell. The host cell containing the haemogregarine becomes stretched and exhibits variable shape and size. The number of parasites present in one host cell is usually one, but frequently two are present, in which case the host cell becomes greatly enlarged and deformed. The intracorpuscular forms while under observation start to turn around in the host cell, and finally breaks through the host cells. Whether this is due to the pressure caused by the cover-glass and by immersion objective or natural phenomenon cannot be determined.

The extracorpuscular stage is gregarine-like in its appearance and movements. The forms found in *Rana pipiens* and *R. clamitans* differ

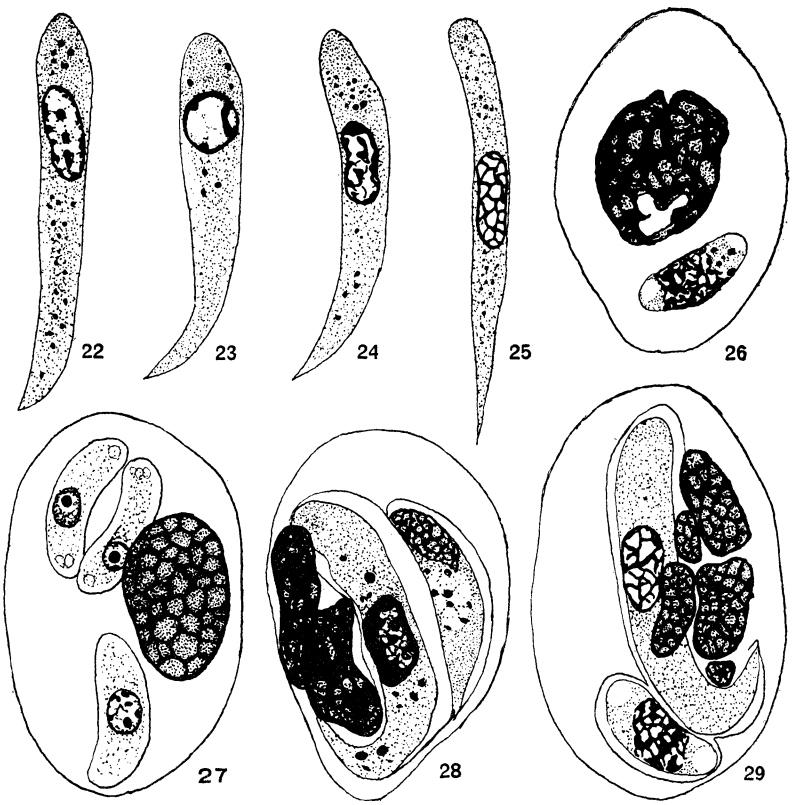
somewhat. The former is shorter and thicker and its anterior end is less truncate with a nucleus situated near the anterior extremity, while the latter is longer and thinner and its anterior end is more truncate with a centrally located nucleus. I have not noticed the difference in living condition, as their length and shape changed from time to time due to the movements. The difference noted in the stained preparations may indicate different circumstances in preparing them, although practically same methods were used in both cases.



Figs. 14 to 21. *Haemogregarina* sp. Fig. 14, a normal red blood corpuscle. Fig. 15, a trinucleate erythrocyte containing an intracellular stage (the structure of one of the nuclei is omitted). Figs. 16 to 18, three infected erythrocytes. Fig. 19, an erythrocyte containing two parasites. Fig. 20, the parasite is just leaving the host cell. Fig. 21, an extracellular giant form still covered with an envelope. Schaudinn: Delafield. x 2100.

The free haemogregarines may be seen usually soon after the preparation was made, but they increase in number in a few minutes. I have often noticed the fact that when a fresh preparation of a small portion of the lung of an infected frog was made, the number of extracorpuscular forms

increased from 1 to 2 to from 12 to 20 in each field (compensation ocular 4 and apochromatic objective 8 mm.) in five minutes while under observation. The body is rounded or truncate at the anterior extremity and tapers to an attenuated posterior end. As was noted by many authors in several species of haemogregarines, the posterior end of the animal is seen connected with a thread-like structure sometimes measuring twice as long as the body. It seems to me that it is a portion of the cytoplasm of the host cell which was in direct contact with the parasite before the latter left it,



Figs. 22 to 29. *Haemogregarina* sp. Figs. 22 to 24, extracellular stages from the lung capillaries of *Rana pipiens*. Fig. 25, an extracellular form from *Rana clamitans*. Figs. 26 and 27, erythrocytes of *Rana pipiens* with small forms. Figs. 28 and 29, erythrocytes with an individual of *Haemogregarina* sp. and a smaller form. Fig. 24, Giemsa; Fig. 27, Heidenhain; the rest Delafield. x 2100.

and which became left behind as the animal moved forward. This view also seems to be reasonable if one considers the fact that the thread-like connection is most conspicuous soon after the parasite leaves the host cell and disappears in a few minutes. The structure of the body in

stained state is similar to that of the intracellular stage. The nucleus assumes sometimes ring form. The average dimensions of forms found in *Rana pipiens:* length 23.8 μ, largest breadth 3.6 μ: and of those found in *Rana clamitans:* length 27.6μ, largest breadth 2.4μ.

Development.—Ordinarily the dimensions of the parasites of both phases described above are somewhat uniform. No stages of division were noted either in the host cell or in free state. Smaller intracellular stages such as shown in Figs. 26 to 29, are often observed. They seem to occur always in the host cells. Oval with a flat or concave and a convex side, they show at each end of the body one to three vacuole-like structures in both fresh and stained conditions. These forms are always present within the same host animal with the large forms described above. But no intermediate stages between them have been seen, although a few forms such as shown in Figs. 28 and 29 are noticed. Without infection experiment, I cannot say whether they are only different stages in the development of one and the same parasite or entirely different forms. Stebbins (1904) considered the smaller form as a distinct species and named it *Haemogregarina catesbianae.* The development of haemogregarines of frogs has not yet been worked out, although *Haemogregarina stepanowi* found an earnest worker in Reichenow (1910) who described interesting observations.

IV *Trypanosoma rotatorium* (Mayer)

Synonyms.—*Paramoecium loricatum* Mayer 1843, *Paramoecium costatum* Mayer 1843, *Amoeba rotatoria* Mayer 1843, *Trypanosoma sanguinis* Gruby 1843, *Monas rotatoria* Lieberkühn 1870, *Undulina ranarum* Lankester 1871, *Paramoecioides costatus* Grassi 1882.

Habitat.—In the blood of *Rana esculenta, R. temporaria, R. clamitans, R. pipiens, R. castebiana, R. galamensis, R. oxyrhynchus, R. mascarensis, Rappia marmorata, Bufo vulgaris, Bufo regularia, Letodactylus ocellatus, Hyla viridis and H. arborea.* A number of host frogs whose specific names were not determined by the original authors are excluded.

The trypanosomes are more numerous in the blood vessels of organs such as liver and especially kidney than in the peripheral or heart blood.

Historical.—Since Gluge (1842) found the organism, several workers noted and studied the flagellate, the chronological review of which is found in Laveran and Mesnil (translated by Nabarro, 1907). Doflein (1910), Lebedeff (1910) and Machado (1911) are more recent conributors to our knowledge concerning this blood parasite of frogs.

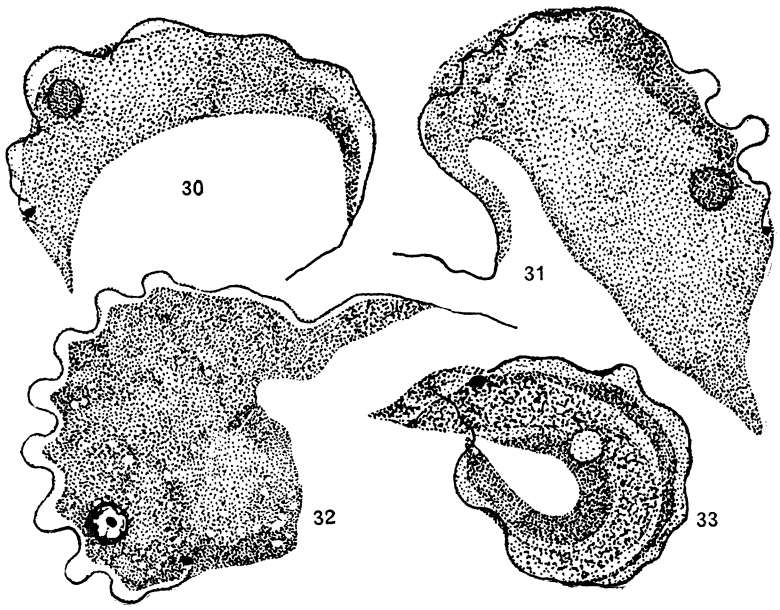
Distribution.—Europe, Africa, Asia, South and North America.

Methods of observations.—The blood should be examined as soon as it is taken from the frog. With a capillary pipette, draw the blood from the frog heart. If it is taken aseptically, the blood can be kept in sterile

condition in test tube with physiological solution and the trypanosomes will live for several days. If one cannot observe the preparation soon after the blood was taken, make temporary hanging drop preparations which may be examined in two to six hours. The trypanosomes are the largest ones known up to date, and its presence can be detected under a low power, although one may have to examine several preparations of the frog blood before finding one individual. When alive one can distinctly see the undulating membrane and the characteristic wriggling movement of the trypanosomes.

To make permanent smears, make ordinary blood smears and fix with either sublimate-alcohol-acetic mixture or with absolute alcohol (for 10 to 20 minutes). Staining with Delafield's haematoxylin, Heidenhain's iron haematoxylin or Giemsa's stain, will bring out morphological details. The nucleus is sometimes hard to stain and prolonged staining is needed to demonstrate its structure.

Morphology.—Polymorphic. Earlier observers held that the difference in size and form among different individuals showed that of the specific characters which view has however been abandoned by modern investiga-



Figs. 30 to 33. *Trypanosoma rotatorium*. Fig. 32, Delafield; rest Giemsa. x 1500.

tors. When the blood is examined soon after its removal from the frog heart, one will see broad forms as well as slender ones mingled with intermediate forms. After some time, some individuals become rounded. The majority have more or less attenuated extremities. The form of the

body changes constantly with the striking movements of the undulating membrane. The flagellum which runs along the outer margin of the undulating membrane is very frequently seen to extend beyond the anterior end of the body. Its length varies, and may not be seen at all. The blepharoplast and nucleus can hardly be seen in actively motile individuals. The cytoplasm is granulated, may contain rounded spaces especially near the posterior margin, and shows in many individuals longitudinal striae.

When stained, the small oval or oblong blepharoplast is seen located some distance from the posterior tip of the body. The flagellum seems to take its origin a short distance from the blepharoplast. The nucleus is located near the blepharoplast and on the same side of the body where the latter is situated. It is rounded and shows its structure poorly with any stain. It contains chromatin granules collected along the periphery. A small karysome may sometimes be seen in the central region. The cytoplasm shows numerous small vacuoles in the posterior half of the body. Dimensions vary considerably. Length, 44 to 70μ and breadth 10 to 35 μ.

Development.—Although artificial cultivation in vitro of *Trypanosoma rotatorium* has successfully been carried out by Bouet, Doflein, Lebedeff and Machado, we know practically nothing concerning its development in the frog body. Some authors such as Laveran and Mesnil, Doflein, etc., have not seen trypanosomes undergoing division in the blood of host frog. My own examination of numerous preparations also leads me to agree with them on this point. On the other hand, França and Athias (1906), Dutton, Todd and Tobey (1907) and Machado (1911) observed stages in division in the smears of frog blood or in preparations of fresh blood of infected frogs "kept aseptically at 72° to 89° F. for two or three days" (Dutton, Todd and Tobey).

According to the observations of the last named three investigators, the trypanosome after losing its flagellum, became rounded and underwent active division, producing numerous small rounded organisms—"forty-one cells were counted, though they were probably more." Each body became ovoid, then pear-shaped, and from the more rounded end a flagellum was produced. These young forms became active and free from the outer covering of the original trypanosome. They divided rapidly by splitting longitudinally increasing in number. They were herpetomonas-like and remained in this condition until the preparations were discarded. The authors studied further stages in stained smears, and stated that the herpetomonas-like forms developed into *inopinatum*-like forms which were also "found in fresh blood, in contradiction to the forms just described, which were found in kept blood alone."

Machado describes stages in division of the trypanosomes in frog. From his statement, it is not clear whether he found these stages in the

blood or kidney. Figs. 14 to 19 and 36 to 40 given by Machado are rather isolated from others and hard to be reasonably connected with the other stages which he figured. I have not had chance of examining *Leptodactylus ocellatus* myself, but comparison of the above mentioned Machado's figures with the vegetative stages of a Myxosporidian, *Leptotheca ohlmacheri* which is described very briefly in this paper and in details in the other paper (Kudo, 1922) and which is not uncommon parasite of the kidneys of *Rana clamitans*, *R. pipiens* and *Bufo lentiginosus* of the United States, leads me to think that the frogs studied by Machado were probably infected by the Myxosporidian or an allied form besides the trypanosomes. Machado states that trypanosomes were abundantly seen in the kidney of the host which fact I also noted. He seems to have mixed the stages of development of a Myxosporidian with those of the trypanosomes.

Judging from the trypanosomes of fishes and reptiles, and *Trypanosoma inopinatum*, another member of the genus parasitic in *Rana esculenta* of Algeria, the present species seems to undergo changes in the body of blood sucking invertebrates. Fuller accounts of the life history of the trypanosome awaits future investigations.

V *Trypanosoma parvum* nov. spec.

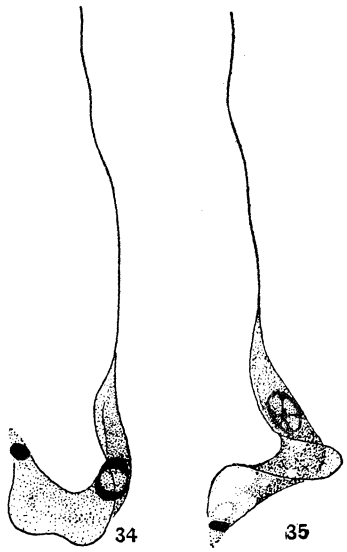
Habitat.—In the blood of *Rana clamitans*. Fourteen specimens were examined between July and September, 1920. In one of them a fairly heavy infection of a trypanosome was noticed. Five to eight active individuals were recognized in every field (compensation ocular 4 and apochromatic objective 8 mm.). The frog also harbored *Trypanosoma rotatorium* in small number (one individual in every two other field under the same combination as noted above), but no haemogregarine was found. I have not seen it since that time, although I have examined about four dozens of *Rana pipens* which were purchased from a Chicago biological supply store.

Methods of observations.—Same as *Trypanosoma rotatorium*. For demonstration the unusually long flagellum, Fontana's staining was used with satisfactory result.

Morphology.—When alive, the movements can be distinguished into two types: travelling and wriggling movements, of which the first is prominent. The active wriggling movements remind one of those of *Trypanosoma lewisi*. The undulating membrane is fairly well developed. The nucleus and belpharoplast are faintly visible, while the relatively long flagellum can distinctly be seen with an oil immersion objective. The cytoplasm contains frequently small rounded clear spaces, and is more or less vacuolated at the posterior portion.

When stained, one finds in them structures typical to a trypanosome. The body is spindle-shaped usually being curved in an arch or S. The

posterior end is ordinarily attenuated and ends in a blunt point, while the anterior extremity is more sharply pointed. The cytoplasm is usually dense from the anterior part to the middle region of the body, while a clear area is frequently seen just posterior to the nucleus, either close to or



Figs. 34 and 35. *Trypanosoma parvum* nov. spec. Fig. 34, Giemsa; Fig. 35, Delafield. x 3300.

somewhat separated from it. The posterior portion is more or less vacuolated as was noted in living specimens. The blepharoplast is located very close to the posterior tip of the body. It is relatively large, and rounded or oblong in shape. The flagellum that borders the outer margin of the undulating membrane does not seem to take its origin directly in the blepharoplast, but arises from a point inconspicuously marked at some distance from the latter. The free portion of the flagellum reaches 15 μ in length, though its length varies most widely. The nucleus is rather large, and is located between the middle and anterior third of the body. It is spherical or oval. In Giemsa stained smears, the peripheral portion stains very deeply, while the central portion is occupied by a few linin threads. A karyosome may sometimes be seen eccentrically located.

Dividing forms were not seen. The trypanosomes are strikingly uniform in size, showing little variation in size and general shape, except the length of the flagellum. Measurements of two hundred specimens in smears fixed with sublimate-alcohol-acetic mixture and stained with Giemsa's solution are as follows: length of body, exclusive the flagellum, 11 to 14 μ, largest breadth including the undulating membrane, 1.2 to 1.9μ, length of free portion of the flagellum 5 to 15 μ.

Of all the trypanosomes of amphibians known up to date *Trypanosoma inopinatum* Sergent et Sergent, 1904, resembles closely to the form just stated. These two forms resemble each other in the dimensions and general resemblance to *Trypanosoma lewisi*. There are however some differences in the location of blepharoplast, the structure of cytoplasm and the general form of the stained individuals which shows the activity of the two forms is not same. The blepharoplast is located more closely to the posterior tip in this form than in Algerian form. The breadth of the American form is 1.2 to 1.9 μ, while that of *Trypanosoma inopinatum* is 3 μ. The cytoplasm of the present form is vacuolated at the posterior portion of the body, while the Algerian form, according to Sergent and Sergent's figures, is uniformly granulated. Furthermore the activity of the two forms appears to be quite different. In the forms I have studied the body shows an arch or S shape in stained smears, while Sergent and Sergent figure more or less straight form, thus indicating probable difference in their activity when alive. Consequently these two forms should better be separated from each other by different specific names, until I am able to compare the preparations of them.

Since the cultivation of *Trypanosoma rotatorium* in vitro has been attempted by Lewis and Williams (1905), the fact that the trypapnosomes undergo division in the culture media resulting in the formation of small spindle-shaped bodies resembling in appearance to Herpetomonas or Crithidia, became known. But in no case, a structure typical to a trypanosome was noted among these small forms. At our present state of knowledge concerning trypanosomes, it is proper for us to consider the extremely small trypanosome described above as independent from *Trypanosoma rotatorium*. As it is morphologically distinguishable from a closely allied form, *Trypanosoma inopinatum*, I propose to name it provisionally *Trypanosoma parvum* nov. spec.

Parasitic flagellates in the intestine

Number of parasitic flagellates have been described in the intestine of frogs. The reader is referred to Dobell (1909) and Swezy (1915, 1915a) concerning them.

VI *Opalina* sp.

The Opalinas described here seem to be identical with *Opalina ranarum* Purkinje et Valentin 1835.

Habitat.—In the rectum of *Rana clamitans* and *R. pipiens*.

Historical.—A complete chronological review of works on Opalinas will be found in Metcalf (1909).

Methods of observations.—The rectum of frog is placed in a small watch glass and opened in physiological salt solution under dissecting microscope. When the preparation is made, the Protozoon will be seen actively moving. In order to retard the active movements, a drop of

two of cherry gum solution may be added. The ciliary movements and the structure of the body can easily be studied. For permanent preparations, follow the methods stated for *Entamoeba ranarum*.

Morphology.—The body is broadly oval, with blunt anterior and more rounded posterior extremities. One side is convex, while the opposite side exhibits a shallow depression at the middle part. The body is highly flattened. Parallel rows of cilia run obliquely. The body is covered with cilia of uniform length. The protoplasm is sharply differentiated into

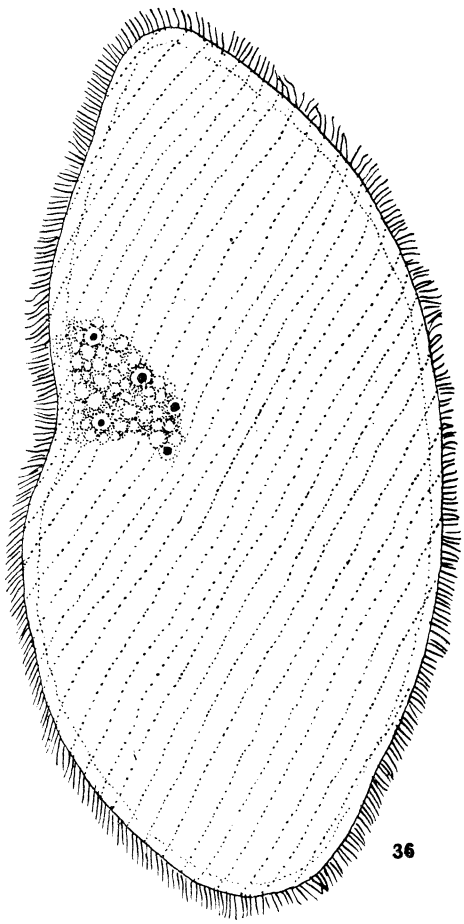


Fig. 36. *Opalina* sp. Delafield. A portion of the body is shown in detail. x 400.

ectoplasm and endoplasm. The ectoplasm is hyaline near the pellicle, but is alveolated near the endoplasm. The latter is granulated in living individuals, but when stained with Delafield's haematoxylin, it shows a vacuolation. The endoplasm contains a large number of nuclei of uniform size. Cytostome or cytpyge is not observed. Dimensions: length 130

to 200 μ, breadth 50 to 120 μ. Occasionally large form reaches 500 μ in length.

Development.—The Protozoon divides in the intestine of the frog, stages of which are commonly seen in the rectum in the summer. I have not studied a new infection of a host frog. According to Neresheimer (1907), *Opalina ranarum* divides successively in the rectum of host frog in the spring, and produces numerous small individuals, each containing a few nuclei. They encyst by producing a hyaline and resistant membrane around them. The cysts come out of the host body with fecal matters and remain on the bottom of the water. When the young tadpoles swallow the cysts, the contents of the latter leave the membrane in the rectum of the new host. The free young opalinas become differentiated into gametes by division and after fusion form zygotes. The zygotes grow into adult ones as the tadpoles metamorphose themselves into adult frogs.

Summary

1) The main object of the present paper is to furnish a brief account of Protozoa parasitic in common North American frogs for general students in Zoology.

2) The occurrence of *Entamoeba ranarum* in *Rana clamitans* is stated.

3) A Myxosporidian, *Leptotheca ohlmacheri* is studied in the kidneys of *Rana clamitans* and *R. pipiens*.

4) *Trypanosoma rotatorium* of *Rana clamitans* and *R. pipiens* is studied.

5) *Haemogregarina* sp. in *Rana clamitans* and *R. pipiens* is studied.

6) A new trypanosome, *Trypanosoma parvum* is described from *Rana clamitans*.

7) *Opalina* sp. from *Rana clamitans* and *R. pipiens* is studied.

8) Methods of observation and brief review of previous works for each of these forms are given.

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